Studies of the Processes Operative in Solutions. XXI.—*The Hydrolysis of Cane Sugar by Dilute Acids.*

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In Part XII of these studies an account was given of a close and extended study of the hydrolysis of cane sugar by means of chlorhydric and nitric acids in aqueous solution and of the influence of corresponding salts on the rate of change. In the course of the investigation, not only were unexpected sources of error discovered in the method used by previous workers in reducing their observations but it became obvious also that the accepted views by which enquirers in this field have been guided hitherto need modification in essential respects. It was pointed out, in particular, that the usual method of applying the mass action equation, to say the least, is not the best: besides a possible general error due to an incorrect initial reading, it gives a spurious constancy to the values of K, making it difficult to detect actual departures from the unimolecular law; it was shown that the following is a better form of equation to use:—

$$\mathrm{K}\log e = \frac{1}{t_2-t_1}\log_{10}\frac{a-x_1}{a-x_2},$$

in which $a-x_1$ and $a-x_2$ are the sugar concentrations at times t_1 and t_2 respectively, successive equal intervals of time being taken as small as the accuracy of the observations allows or the rapidity of the change demands.

In the investigation referred to, the hydrolytic activity of the acid studied was determined in solutions of moderate strength; consequently it was desirable to consider whether the conclusions arrived at by previous workers who have used very dilute acids may not also require modification. The subject has been dealt with already in Part VI of the "Studies on Enzyme Action" published from the laboratory of the City and Guilds College,[*] by E. F. Armstrong and R. J. Caldwell, whose experiments were made directly with the object of contrasting the action of acids and enzymes at low degrees of concentration. As it had been found that when only a very small proportion of enzyme was present the course of the change approximated to a linear and not to a logarithmic function of the time, it appeared not unlikely that the same result would follow in the case of acids. Armstrong and Caldwell used solutions of M/500 strength of hydrogen chloride and in order that hydrolysis might be about half completed within 10 hours carried out their experiments at about 40°. The conclusion at which they arrived was that though the results they obtained did not provide evidence that the change proceeds at a strictly linear rate, the approximation to a straight line law during the first two and a half hours is very close, the first 10 or 15 per cent. of the change being practically linear. They therefore regarded their results as proof that the hydrolytic action of a small proportion of an acid is comparable with that of a small proportion of an enzyme.*

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[*] 'Roy. Soc. Proc.,' 1904, vol. 74, p. 195.

Assuming this conclusion to be correct, it was hoped that by making a series of experiments and beginning with the concentrations at which the rate of change was in accordance with the mass action law, subsequently increasing the ratio of sugar to acid, it would be possible to find the dilution at which the rate of change began to be a linear function of the time.

As the improvements that have been made, from time to time, in the apparatus permit observations being carried on during many days at a constant temperature, the experiments were all carried out at 25° C. The hydrolyst used was dilute sulphuric acid.

In the first experiment, one-tenth of a molecular proportion of acid was used with 200 molecular proportions of water and one of sugar. Readings were taken 15 minutes and 30 minutes after mixing and then at intervals of 60 minutes, during 7 hours. The solutions were mixed at 10.45 A.M. The results are given in Table I. Each reading is a mean of five observations taken at intervals of 30 seconds at the time stated.

The first difference is low probably because the temperature in the tube was slightly low at first. It will be seen that after the first two intervals, when but a small fraction of the sugar is inverted, the rate of change decreases regularly as the action proceeds and that there is no indication of a linear relationship.

In a second experiment, the concentration of the acid was reduced to one-hundredth of a molecular proportion. The rate of change was very slow.

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* In view of the importance of the conclusions which E. F. Armstrong and R. J. Caldwell were inclined to draw from their observations on the action of acids on cane sugar in dilute solutions, it appeared to me to be necessary to verify their results, utilising for this purpose the improvements effected by Mr. Worley in the polarimetric method and also in reducing the observations. I am indebted to Mr. Worley for having undertaken the somewhat arduous task. I may add that he completed the work last summer and that a preliminary verbal communication of the results was made to the Biochemical Club at their meeting on July 8, 1911.—H. E. A.

The observations were continued—with one or two interruptions caused by accidents to the temperature regulator—during 13 days. The results are given in Table II. Again in this case the rate of change decreased as the action went on. As the end-value was not determined, the value of K could not be calculated. On account of the interruptions, the time is given as the interval between successive readings.

Table I.

Time in minutes.	Rotation.	Differences per 60 minutes.
15	26 ·412	0 ·522
35	26 ·238	0 ·570
95	25 ·668	0 ·571
155	25 ·097	0 ·564
215	24 ·533	0 ·554
275	23 ·979	0 ·547
335	23 ·432	0 ·532
395	22 ·900	0 ·528
455	22 ·372	

Table II.

Time intervals in hours.	Rotation.	Differences per 60 minutes.
4 ·0	26 ·438	0 ·069
	26 ·163	
3 ·5	24 ·835	0 ·069
	24 ·595	
12 ·0	22 ·658	0 ·064
8 ·5	21 ·885	0 ·063
21 ·0	21 ·353	0 ·060
19 ·0	20 ·102	0 ·058
5 ·0	18 ·992	0 ·058
	18 ·703	
6 ·0	17 ·650	0 ·055
25 ·5	17 ·317	0 ·052
23 ·5	15 ·980	0 ·051
24 ·5	14 ·780	0 ·048
26 ·5	13 ·610	0 ·047
38 ·5	12 ·363	0 ·043
	10 ·684	

In Experiments 3 and 4 the concentration of the acid was kept the same as in the previous experiment, viz. one-hundredth of a molecular proportion, whilst that of the sugar was increased to two and four molecular proportions respectively. The two experiments were carried out simultaneously on the two water circuits of the apparatus and were continued during a week with one interruption near the beginning. The end values were obtained by heating a given weight of the original solution in a steam oven and making up by addition of water any loss caused by evaporation; they are at the best only approximate. The great difficulty of obtaining correct end values has been already dealt with* and will be further discussed in a later paper. Two-decimetre tubes were used in these experiments in place of the four-decimetre tube used in the previous experiment. The results are tabulated in Tables III and IV.

In the third columns are given the differences per 60 minutes derived from the consecutive readings; the fourth contain the values of the velocity constant deduced in accordance with the formula

$$K = 10^7 \frac{1}{t_2 - t_1} \log_{10} \frac{a - x_1}{a - x_2},$$

the interval of time $t_2 - t_1$ being the interval between the consecutive readings from which the values $a - x_1$ and $a - x_2$ are derived.

The differences per 60 minutes rise at first but the abnormally high value of the third difference in both experiments would seem to indicate an unobserved temporary rise in the temperature during part of that interval. In any case, after the third difference, when only about one-eighth of the sugar had been changed, the fall in the value of the differences is obvious.

Table III.

Time in minutes.	Rotation.	Differences per 60 minutes.	K.
5	26·519		
60	26·467		
1080	25·342	0·066	144
1865	24·478		
2790	23·448	0·067	155
3880	22·198	0·068	163
5410	20·671	0·060	149
6820	19·250	0·061	158
8260	17·828	0·059	165
9700	16·406	0·059	172
Complete change	−7·642		

Table IV.

Time in minutes.	Rotation.	Differences per 60 minutes.	K.
15	47·974		
60	47·892		
1140	45·680	0·123	146
1950	43·992		
2860	42·148	0·122	155
3960	39·806	0·128	169
5490	36·958	0·112	155
6900	34·362	0·110	162
8340	31·823	0·106	164
9780	29·322	0·104	169
	−13·790		

In the fourth column in the tables the values of K fluctuate to some extent, on account of the errors of reading due to the very small amount of change during each interval. They rise as the action proceeds but the fact that practically the same value is obtained in the two experiments is absolute proof that the rate of change, at these concentrations, is proportional to the amount of cane sugar in solution. The experiments show that at the relative concentrations of sugar and acid considered there is no indication of the action being a linear function of the time.

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* Part XII, 'Chem. Soc. Trans.,' 1911, p. 349.

It now became necessary to consider whether the gradual rise in the values of K deduced by Armstrong and Caldwell were not due to the method of reducing the experimental values which they adopted.

The values of K were deduced in the manner almost universally followed by means of the equation

$$\mathrm{K} = \frac{1}{t}\log\frac{a}{a-x}.$$

As has been pointed out, this method is open to serious objections. It gives a spurious constancy to K and any error in the value of a affects all the separate values of K unequally, those at the beginning being most affected. In the four experiments considered, a was determined 10 or 15 minutes after the solutions had been run into the polarimeter tubes. If, as is very possible, the temperature had not then attained its correct value but was somewhat low, an apparent rise in the early values of K and their constancy later on was to be expected from this cause alone. The values of K have consequently been recalculated using the equation

$$\mathrm{K} = \frac{1}{t_2-t_1}\log\frac{a-x_1}{a-x_2}$$

and keeping the interval of time t_2-t_1 constant.

The results of recalculating the values given by E. F. Armstrong and Caldwell in the case of two of their experiments (representative of the two concentrations examined) are set forth in Tables V and VI, along with their own values, for comparison. The strength of the acid was M/500 in each case; the sugar solutions contained 171 and 342 grm. of cane sugar respectively per litre. In Table V the interval of time adopted is 90 minutes and 60 minutes and in Table VI 60 minutes. A comparison of the numbers arrived at with those previously obtained by Armstrong and Caldwell shows very clearly the errors that may arise from applying the mass action equation in the way usually advocated. In the first place, the marked rise (on which Armstrong and Caldwell relied) in the early values and constancy in later values disappears and there is apparently a small continuous rise throughout each series which is scarcely perceptible in Table VI. The rise is most probably caused by an error in the final reading at the completion of the inversion. In the second place, the actual values of K obtained by the two methods are somewhat different and the

Table V.

Time in minutes.	Rotation.	K.		
		Armstrong and Caldwell.	*t* = 90.	*t* = 60.
0	22·22	—	449	429
15	21·83	412	442	453
30	21·40	437	441	436
45	21·00	437	442	419
60	20·63	430	459	469
75	20·18	445	447	453
90	19·77	449	453	445
105	19·45	438	474	461
120	19·00	449	450	454
135	18·63	448	455	467
150	18·27	447	449	455
165	17·92	446	465	465
180	17·52	451	462	440
195	17·18	449	469	448
210	16·83	450	—	472
225	16·47	451	—	487
240	16·17	448	—	—
255	15·78	453	457	—
270	15·43	454	463	—
285	15·05	459	445	431
300	—	—	—	—
315	—	—	—	—
330	—	—	—	—
345	13·87	455	458	474
360	13·57	454	460	453
375	13·25	455	453	449
390	12·95	456	450	448
405	12·65	457	443	443
420	12·42	454	461	462
435	12·13	454	450	452
450	11·85	453	—	455
465	11·58	455	—	440
480	11·32	455	—	—
495	11·07	454	—	—
510	10·80	455	—	—
525	10·58	453	—	—
Complete change	−5·37			

Table VI.

Time in minutes.	Rotation.	K.	
		Armstrong and Caldwell.	*t* = 60.
0	46·03	—	479
10	45·47	428	485
20	44·83	461	490
30	44·20	471	491
40	43·62	468	485
50	42·97	478	489
60	42·37	479	493
70	41·80	477	495
80	41·17	482	501
90	40·57	485	499
100	40·07	488	516
110	39·45	483	507
120	38·85	486	513
130	38·28	487	511
140	37·67	491	494
150	37·13	490	506
160	36·55	492	498
170	36·03	491	502
180	35·43	495	491
190	34·93	494	—
200	34·45	492	522
210	33·87	495	512
220	33·38	494	505
230	32·87	494	511
240	32·38	494	499
250	—	—	—
260	31·28	499	506
270	30·80	499	514
280	30·38	496	534
290	29·87	498	531
300	29·48	495	542
310	28·83	502	509
320	28·42	500	526
330	27·93	501	515
340	27·43	503	497
350	26·97	503	502
360	26·55	503	503
370	26·12	503	503
380	25·65	504	505
390	25·25	504	505
400	24·87	502	519
410	24·42	503	526
420	24·02	503	532
430	23·62	503	538
440	23·17	504	525
450	22·80	503	532
460	22·38	504	533
470	21·93	506	510
480	21·53	507	536
490	21·13	507	—
500	20·77	507	—
510	20·40	507	—
520	20·00	508	—
530	19·68	506	—
540	19·27	508	—
Complete change	−11·12		

irregularity of those obtained on using a constant time interval in the calculations shows that no importance can be attached to variations in the value of the constant when the interval is less than 60 minutes. The first five or six values of K, as originally determined in each series, consequently are not to be relied upon. All evidence of a linear rate thus disappears completely. The most conclusive evidence against it, however, is obtained on comparing the values of K for solutions of different proportions of sugar. If, as Armstrong and Caldwell supposed, there be a tendency towards a linear change in the more dilute sugar solution, *i.e.*, that the rate of change at this concentration tends to be independent of the concentration of the sugar, then increase in the concentration of sugar should not increase the rate of change in the same proportion and the value of K should be much smaller in the case of the more concentrated sugar solutions.

In point of fact, in the more concentrated solution, the value of K, instead of being less, was greater than in the more dilute.

It therefore appears that there is no reason to suppose that the rate of change conditioned by a dilute solution of an acid differs in the manner suggested by Armstrong and Caldwell from that effected by more concentrated acid solutions. Consequently, there is not, at present, any evidence to show that the action of dilute acids is analogous to that of an enzyme used in small proportion. This conclusion, however, in no way affects the conclusion on which Armstrong and Caldwell's experiments were based, that a constant weight and not a constant proportion of the total amount of cane sugar present is hydrolysed in unit time when only a very small proportion of *enzyme* is used: the evidence adduced by Adrian Brown in support of this conclusion being decisive and in no way subject to the criticism which may be applied to the results obtained with acids.

Such a conclusion serves to accentuate the marked difference in behaviour of enzymes and of acids as hydrolytic agents.

The above account was written before the publication by Messrs. M. A. Rosanoff, R. H. Clark and R. L. Sibley* of the results of "A Reinvestigation of the Velocity of Sugar Hydrolysis." These observers attribute the fall in the velocity coefficient found by Julius Meyer and the rise found by E. F. Armstrong and Caldwell to an erroneous value having been assigned to the initial rotation; they show that values of the constant are obtained that neither rise nor fall by substituting for the observed initial values those

* 'Amer. Chem. Soc. Journ.,' Dec., 1911, p. 1911.

deduced from the subsequent part of the change. They therefore conclude that the hydrolysis follows a strictly unimolecular course.

There is little doubt that in Julius Meyer's experiments,* as Hudson has already pointed out,† the fall in the value of the constant arrived at is due to errors in the initial values; the rise observed by Armstrong and Caldwell is doubtless due to a similar cause. These errors, however, are attributable probably not to inaccurate observations of the rotation but to the fact that the temperature had not become constant.

Although Rosanoff, Clark and Sibley have arrived at a right conclusion, they have done so by an unsound method, by calculating an initial value in correspondence with the assumption they set out to prove, namely, that the action proceeds at a strictly unimolecular rate from the very start. They have also overlooked the fact that Armstrong and Caldwell's values at time 0 are not the rotations at the time of mixing but those observed 10 or 15 minutes later; it is, in fact, distinctly stated by them that the first reading was taken 10 or 15 minutes after mixing, when the temperature had attained to that of the thermostat.

The American observers say "that Armstrong and Caldwell's observed values of α_0 are erroneous and that calculated values are much more reliable is clearly indicated by the data reproduced in Tables V and VI. The solutions corresponding to these tables have identically the same final rotation, $-5{\cdot}37°$, hence must have been identical in composition. Yet the observed initial rotations were respectively 22·22° and 22·12°. On the other hand, our calculated values, 22·28 and 22·26, are practically equal."

The difference in Armstrong and Caldwell's values at time 0 is obviously due either to a slightly different interval of time having elapsed between mixing and taking the first reading or else to the temperature at mixing not having been exactly the same in both experiments.

Rosanoff, Clark and Sibley have used the equation

$$\mathrm{K} = \frac{1}{t}\log\frac{a}{a-x},$$

which is so generally adopted and have not seen that the errors due to an erroneous initial observation may be avoided by using the equation

$$\mathrm{K} = \frac{1}{t_2-t_1}\log\frac{a-x_1}{a-x_2}.$$

They have also ignored the possibility of an error in the final rotation α_∞. In Part XII (*loc. cit.*) it is shown that the observed end point may be erroneous to a considerable extent; it is also pointed out that it is possible to deduce an end point which gives the appearance of constancy to the values of K, when in reality they should show a steady rise or fall. The argument may also be applied when the values of K are deduced by using the equation

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* 'Zeit. Physik. Chem.,' 1908, vol. 62, p. 59; *ibid.*, 1910, vol. 72, p. 117.

† 'Amer. Chem. Soc. Journ.,' 1908, vol. 30, p. 1165; *ibid.*, 1910, vol. 72, p. 17.

$$K=\frac{1}{t}\log\frac{a}{a-x}$$

in the case of a calculated initial value a, as it is possible to so adjust the initial value as to make the values of K approximate to constancy, although in reality they should rise or fall.

Studies of the Processes Operative in Solutions. XXII.—*The Hydrolysis of Cane Sugar by Sulphuric Acid; also a Note on Improvements in Polarimetric Apparatus.*

By F. P. Worley.

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In view of the fact that the acids used in the inquiries discussed in Part XII and the succeeding parts of these studies were all monobasic, it was desirable to investigate the hydrolytic activity of a dibasic acid, more especially with a view to following the progressive alteration in molecular hydrolytic activity and apparent degree of hydration attending changes of concentration. A series of experiments, using sulphuric acid as the catalyst, has been carried out therefore on the lines of those described in Part XII. Some of the complexities of the hydrolytic process and of the polarimetric method of following its course were dealt with in that communication; moreover, several disturbing factors were referred to, such as the change in the osmotic conditions as the action proceeds and changes in the optical activity of the various substances due to dilution, to their mutual interference, and to the influence of acids or salts; the possible influence of "mutarotation," however, was not mentioned, as there appeared to be no reason to believe that it in any way affected the results.

The study of the rates at which hydrolysis is effected by sulphuric acid of different degrees of concentration has shown that exactly the same complexities are met with in this case as were experienced in the case of